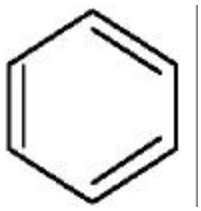
Benzene

CAS Registry Number 71-43-2



I. Physical and Chemical Properties

Description	clear, colorless liquid
Molecular formula	C_6H_6
Molecular weight	78.11 g/mol
Air concentration conversion	1 ppm = 3.19 mg/m^3 (at 25^{0}C)

II. Overview

Exposure to benzene is associated with increases in numerous adverse effects including bone marrow damage, changes in circulating blood cells, developmental and reproductive effects, alterations of the immune system, and cancer. Benzene is absorbed through all routes of exposure, and the metabolism and distribution does not appear to depend significantly on route of exposure (OEHHA, 2000a). In humans, the most sensitive responses to benzene are those related to the blood-forming organs.

In California, the acute Reference Exposure Level (REL, 6 hr) for benzene is 1300 µg/m^3, and the chronic Reference Exposure Level (REL) is 60 µg/m^3 (OEHHA, 1999a; 2000b). The acute and chronic REL values are based on hematotoxicity among benzene-exposed workers. The cancer potency factor is 0.1 (mg/kg-day)$^{-1}$, which corresponds to a unit risk factor of 2.9 x 10^{-5} (µg/m^3)$^{-1}$ (OEHHA, 2001). The California TAC value is based on increased rates of leukemia among benzene-exposed workers.

Benzene causes reproductive and developmental effects including reduced fetal weight, delayed ossification, fetal chromosomal damage, altered fetal hematopoiesis, and alterations to sperm. OEHHA

(1997) extensively reviewed the available literature on benzene's reproductive and developmental toxicity. Some of the conclusions of that document will be discussed below. Benzene was listed in 1997 as a reproductive and developmental toxicant by the State of California under Proposition 65, the Safe Drinking Water and Toxic Enforcement Act of 1986. OEHHA is currently developing a Maximum Allowable Daily Level (MADL) for the developmental effects of benzene as part the Proposition 65 Program.

However, since cancer is still likely to "drive" the TAC toxicity number for benzene, the focus of this summary will be to describe the scientific evidence on whether exposures to benzene early in life would result in a greater carcinogenic impact than exposures occurring later in life.

Summary of potential for differential effects

Benzene has been implicated as a potential risk factor for the development of childhood leukemia (OEHHA, 1997; Smith and Zhang, 1998; U.S. EPA, 1998; Reis et al., 1999). Some epidemiological studies have reported statistically significant associations of increases in childhood leukemia, especially acute non-lymphocytic leukemia, with maternal exposures during pregnancy or paternal exposures prior to conception to benzene or benzene-containing mixtures (Shu et al., 1988; Buckley et al., 1989; McKinney et al., 1991). These findings are consistent with evidence in animals that exposure to benzene induced DNA damage to sperm, transplacental genotoxicity, transplacental altered hematopoiesis and, possibly, transplacental carcinogenicity. However, other epidemiological studies did not find an association between occupational paternal exposure to benzene and childhood leukemias (Shaw et al., 1984; Kaatsch et al., 1998; Shu et al., 1999; Feychting et al., 2001).

Also, there is evidence in animals that exposures to benzene early in life and through adulthood resulted in a 2-fold higher increase in the incidences of cancer compared to exposures only as adults (Maltoni et al., 1989).

Since benzene has been associated with childhood leukemia in several epidemiological studies, and since early life exposures appear to differentially increase lifetime cancer risk in animal studies, benzene

is considered to be a priority chemical for evaluation of potential differential effects on infants and children.

III. Principal Sources of Exposure

Except for cigarette smoking, vaporization of gasoline and automobile exhaust are the primary sources of benzene exposure in the general population (Wallace, 1996). Past formulations of gasoline contained about one to two percent benzene; however, current formulations are required to contain no more than one percent benzene by volume.

The California Air Resources Board (CARB) routinely monitors ambient air concentrations of benzene throughout California through its air toxics network. In 1982, when the monitoring program began, estimates of the population-weighted annual concentration of benzene were roughly 5 ppb (16 $\mu g/m^3$) (CARB, 1984). These concentrations have declined steadily over time such that in 1994 average estimates across the state were approximately 1.2 ppb (3.8 $\mu g/m^3$) (CARB, 1995). The 12-month average ambient air concentration of benzene for California in 1997 to 1999 was 0.85 ppb (CARB, 2000). In addition to benzene emissions derived from mobile sources, there are significant emissions of benzene from stationary sources in California. These were estimated to be at least 870,000 pounds per year in 1997, based on data reported under the Air Toxics "Hot Spots" Program (AB 2588) (CARB, 1997).

In studies of human exposures to benzene, the primary sources of exposure among non-smokers were auto exhaust and gasoline vapor emissions. Most of the benzene in outdoor air comes from auto and gasoline vapor emissions; inhalation of ambient air accounts for a large percentage of an individual's total benzene exposure. Also, indoor air exposures due to intrusion of evaporative gasoline fumes in homes with attached garages and personal activities such as driving can contribute significantly to an individual's total exposure to benzene (Wallace, 1996). For example, a study sponsored by CARB found that benzene concentrations inside a vehicle was 3-to 7-fold higher than ambient levels nearby

(CARB, 1998). Other sources of exposure to benzene include contaminated drinking water, which can arise for example from contamination of water sources by leaking from underground fuel storage tanks.

IV. Potential for Differential Effects

The evidence presented here will be divided along two lines:1) evidence that suggests that benzene may be a transplacental and preconceptional carcinogen associated with childhood leukemia; and 2) evidence to suggest that children or the developing fetus exposed to benzene may exhibit a higher lifetime risk of cancer than equivalent exposures to adults.

A. Summary of Key Human Studies: Cancer

All major authoritative bodies classify benzene as a known human carcinogen, based on increased rates of leukemia among different benzene-exposed human populations. However, there is some evidence to suggest that benzene also causes childhood leukemia (see key human studies below), although a causal relationship would be difficult to establish at this time. Many researchers contend that childhood and adult leukemias are different diseases with different etiologies.

a) Childhood exposures resulting in increases in adult-onset leukemia

There are no human studies available that have examined childhood exposures to benzene and increases in lifetime risk of cancer.

b) Benzene and childhood leukemia

Benzene has been implicated as a potential risk factor for the development of childhood leukemia (OEHHA, 1997; Reis et al., 1999; Smith and Zhang, 1998; U.S. EPA, 1998). Some large epidemiological studies have reported increases in childhood leukemia associated with *in utero* exposures, and paternal exposure prior to conception to benzene. However, other studies do not suggest an association. The studies and their strengths and limitations are discussed below.

Childhood cancer is the second biggest killer of children (the first being accidents), and the most common form of childhood cancer is leukemia (Sandler and Ross, 1997). Moreover, childhood cases through 14 years of age account for 12 percent of all leukemia cases in the U.S. (Sandler and Ross, 1997). The incidence of leukemia among children younger than 15 years of age has remained fairly constant over the past 20 years except for a sharp increase from 1983 to 1984, which likely reflects a change in diagnosis or reporting (Linet et al., 1999). Deaths rates from childhood leukemia have declined steadily since 1975, which is believed to be due to increased survival from medical advances in treatment (Linet et al., 1999). Exposures to carcinogens during *in utero* development and in early childhood have been suggested as possible causal factors responsible for some of the increases in leukemia (Reis et al., 1999). In adults the most common leukemia types are myeloid and lymphatic, whereas the predominant type of leukemia in children is lymphatic. Benzene exposure in adults is most strongly associated with acute myelogenous leukemias (AML), although increased risks of non-AML leukemias are also reported (Crump, 1994; Hayes et al., 1997). Likewise, some epidemiological studies that have examined childhood leukemias by subtype with respect to paternal or maternal exposures to benzene have also found the strongest associations with acute myelogenous leukemias (Shu et al., 1988; Buckley et al., 1989).

c) Paternal or maternal exposure to benzene and childhood leukemia

A large case control study reported finding a statistically significant association, including a trend in exposure duration, between paternal benzene exposure and childhood acute non-lymphocytic leukemia among progeny (Buckley et al., 1989), while one study with a smaller number of cases did not (Kaatsch et al., 1998). Additionally, a study examining paternal benzene exposure and childhood leukemia (not separated by subtype) reported a positive association (McKinney et al., 1991). Two studies of paternal benzene exposure prior to conception and childhood leukemia (not separated by subtype) (Shaw et al., 1984; Feychting et al., 2001) or acute lymphocytic cases only (Shu et al., 1999) did not find an association. With respect to maternal exposure to benzene, high relative risk estimates have been reported for benzene exposure and childhood acute non-lymphocytic leukemia among progeny in one report (Shu et al., 1988), while a separate report did not find an association (Kaatsch et al., 1998).

Studies of childhood acute lymphocytic leukemia, the most common childhood leukemia subtype, did not find an association with maternal benzene exposure (Kaatsch et al., 1998; Shu et al., 1999). Other studies of parental exposures to childhood leukemia and benzene (among other agents) were also identified (Lowengart et al., 1987; Feingold et al., 1992) but the numbers of cases (or numbers of benzene-exposed parents) were too small to provide any meaningful information.

Shaw et al. (1984) examined the association between disease and risk factors including maternal age, birth order, socioeconomic status, and paternal occupation in a matched case-control study evaluating 255 cases of childhood leukemia reported to the California Tumor Registry. Controls (N=510) were matched by sex and county, by selecting the birth certificate immediately preceding and following the case's birth certificate. Exposure was determined from the occupation of the father as listed on the birth certificate. Occupational classifications determined by the NIOSH National Occupational Hazard Survey 1971-74 were used to classify fathers in the study as "potentially exposed" or "not exposed". This study found no association between paternal benzene exposure and childhood leukemia. As noted by the authors, it is possible that the failure to detect an association in this study is due to misclassification of exposure status. A limitation of the study is the likelihood of multiple chemical exposures confounding the data.

Shu et al. (1988) examined the association between maternal and paternal occupational exposures during pregnancy and childhood leukemia in a well-designed matched case-control interview study in Shanghai, China. Using a population registry, 309 childhood leukemia cases in China were compared to 618 control children. Exposure were ascertained through personal interviews with the parents which inquired about occupational exposures, and history x-rays, drug use, diseases and other potential risk factors. Paternal occupation during pregnancy was not associated with childhood leukemia, and exposures prior to conception were apparently not ascertained. These investigators found a statistically significant association between childhood leukemia and maternal occupation in the chemical industry (chemical processors and related workers, rubber and plastic products makers, leather workers, painters, and chemical analysts) (OR 3.3, 95 percent CI = 1.6 to 6.8). They found suggestions of increased risks associated with self-reported occupational exposure to benzene (OR 2.0, 95 percent CI

= 0.9 to 4.3) and gasoline (OR 1.6, 95 percent CI = 0.8 to 3.1). When childhood leukemia cases were separated by histopathological cell type, maternal benzene exposure was found to be associated with statistically significant increased risks of acute non-lymphocytic leukemia (OR 4.0, 95 percent CI = 1.8 to 9.3) but not with acute lymphocytic leukemia. Maternal gasoline exposure was associated with an increased risk of acute lymphocytic leukemia (OR 1.7, 95 percent CI = 1.0 to 3.0).

Buckley et al. (1989) conducted a case-control study of paternal and maternal occupational exposure to benzene of 204 children, aged 18 or less, in the U.S. with acute non-lymphocytic leukemia. 204 controls were also examined which were identified by random digit dialing and were matched by date of birth and race. Exposures were assessed through a one hour questionnaire with the mother and father. An elevated association between acute non-lymphocytic leukemia and occupational exposure of the fathers to solvents (including benzene) was observed. Odds ratios (OR) for childhood leukemia and paternal exposure to solvents relative to fathers with no solvent exposure were OR=2.6 (95 percent CI 1.3-5.5) for 1-1000 days exposed and OR=2.0 (95 percent CI 1.2-3.8) for fathers exposed for more than 1000 days. Similar associations were observed for childhood leukemia and paternal exposure to petroleum products (OR 2.4 for prolonged exposure, 95 percent CI = 1.3 to 4.1, p-value for trend = 0.002). This study is limited by the possibility of recall bias, although the authors believed that this was not likely to be occurring. Also, exposure groups included multiple chemicals of which benzene was only a part. One strength of the study for the question at hand is that it focused exclusively on acute non-lymphocytic leukemia, the subtype that is most strongly associated with adult exposures to benzene. Data on maternal exposure to solvents were not reported.

In another matched case-control study, McKinney et al. (1991) evaluated the associations between self-reported parental exposures to specific agents and childhood leukemia and non-Hodgkin's lymphoma in three areas of England with previously documented high rates of these diseases. Children diagnosed with leukemia or non-Hodgkin's lymphoma in the study area between 1974 and 1988 were included in the study. Cases occurring during this period included 113 cases of acute lymphoblastic leukemia (75 percent), 21 other cases of leukemia (14 percent), and 17 cases of non-Hodgkin's lymphoma (11 percent). Each case was matched to two controls by sex, date of birth, and health district of birth. Cases were included in the analysis if data were available for the case and at least one control. Exposure data were collected through face-to-face home interviews that asked questions specifically about parental exposure at work or through hobbies to a variety of suspected toxicants.

Twelve of 101 cases (12 percent) compared to six of 178 controls (three percent) had fathers who reported preconception exposure to benzene (OR 5.81, 95 percent CI = 1.67 to 26.44). Of all the specific agents examined, "the only independent contributions to risk in the preconceptional period were exposures to wood dust (odds ratio 3.00, 1.50 to 5.90), radiation (2.94, 1.13 to 7.63), and benzene (4.82, 1.24 to 18.84)." This study is limited by the possibility of recall bias.

Kaatsch et al. (1998), in a case-control study, examined the associations of various risk factors for 1037 cases of acute lymphocytic leukemia, 147 cases of non-lymphocytic leukemias and 234 cases of non-Hodgkin's lymphoma in Germany. Cases were identified through the German Childhood Cancer Registry. One control for each case was identified and recruited through local registration offices. Controls were matched by age, sex, and place of residence at the time of diagnosis. Exposure information (including benzene exposure) was obtained through self-administered questionnaires and subsequent telephone interviews by trained interviewers. Response rate for both the questionnaire and the telephone interview were different for cases and controls. For example, questionnaire response rates were 81.1 percent for cases and 66.6 percent for controls. Although no data were provided, the authors noted that they did not find any association between parental benzene exposure and childhood leukemias. The authors stated in their methods section that they analyzed the data by leukemia subtype using conditional logistic regression, but no benzene-related results by subtype were presented. Kaatsch et al. (1998) found no associations between exposure to ionizing radiation and childhood leukemia, and reported a significant negative association between maternal smoking and childhood leukemia. The study may be limited by response bias, which the authors did not address in the discussion of the findings. As with the other studies, this study is limited in that multiple chemical exposures of the parents are potential confounders. A strength of the study is the large number of subjects examined.

In a case-control study, Shu et al. (1999) examined 1984 case of child acute lymphocytic leukemia in the U.S. and Canada, identified through the Children's Cancer Group. Controls (N=1986) were selected by random digit dialing, matched by age, race and area code. Exposure information was obtained through a questionnaire and telephone interviews with the mother and also with the father if available. Paternal or maternal exposure to benzene or "petroleum products," either prior to

conception, during pregnancy or postnatal was not associated with childhood acute lymphocytic leukemia. As with the studies described above, this study is limited in that multiple chemical exposures of the parents are potential confounders. The numbers of cases assessed in the study is high; however, the study was limited to acute lymphocytic leukemias. Benzene is most commonly associated with acute non-lymphocytic leukemias from occupational exposures as adults (ATSDR, 1997). Also, Shu et al. (1988) suggested that benzene exposure *in utero* was more strongly associated with childhood non-lymphocytic leukemia than childhood lymphocytic leukemias. Thus, limiting the focus of the study to acute lymphocytic leukemias, as was done by Shu et al. (1999), may reduce the ability to observe an association with benzene exposure.

Feychting et al. (2001) examined 161 leukemia cases as part of a larger cohort study of Swedish children born to married couples in 1976-77 or 1981-82 (N=235,635 births). All children were followed through 15 years of age, and their vital status was determined through the Swedish Cause of Death Registry. Exposures to the father were obtained from the father's occupation as listed on the 1975 census (for births occurring in 1976 or 1977) or on the 1980 census (for births occurring in 1981 or 1982). The father's occupation was linked to a job-exposure matrix constructed as part of the study by industrial hygienists. Two occupational hygienists assessed the probability of exposure to different agents based on the type of industry and job title. Benzene was one of many specific compounds considered. Benzene was not significantly associated with paternal exposure prior to conception (RR = 1.23, 95 percent CI 0.39-3.85). The study strengths included the cohort study design which does not have the potential for recall bias and large numbers of children considered, although the total number of leukemia cases was moderate (N=161). Potential limitations of the study include significant possibility for exposure misclassification. Also, the study did not examine associations of benzene exposure with leukemia subtype.

Benzene is a component of gasoline and diesel fuels and engine exhaust; thus, workers in occupations closely related to motor vehicles are expected to be exposed to benzene. Researchers from the National Cancer Institute published a review of the epidemiological studies of childhood leukemia and paternal exposures via occupations involved with motor vehicles or exhaust gases (Colt and Blair,

1998). They summarized the evidence as follows: "There have been 12 studies of childhood leukemia and paternal employment in occupations related to motor vehicles or involving exposure to exhaust gases. Elevated risk was found in most of these studies, with statistically significant findings in six. Significant associations were found among diverse occupations such as motor vehicle or lorry drivers (12,32), mechanics and gas station attendants (17, 27, 33), and broader groups of motor vehicle-related occupations (18). In their review of leukemia, Linet and Cartwright (30) suggested that the link between motor vehicle occupations and adult leukemia may be due to benzene and other components in engine exhausts" (Colt and Blair, 1998). As with the case-control studies described above, this database provides suggestive evidence of an association between parental exposure to benzene and childhood leukemias.

Associations of paternal exposures and childhood leukemia are consistent with observations in animals that benzene induces DNA damage in sperm (see below). Also, associations of maternal exposures and childhood leukemia in humans are supported by observations in animal studies that indicate that benzene crosses the placenta and induces DNA damage in the fetus (see Section IV.B. Summary of the Key Animal Studies).

d) Other Critical Information from Human Studies

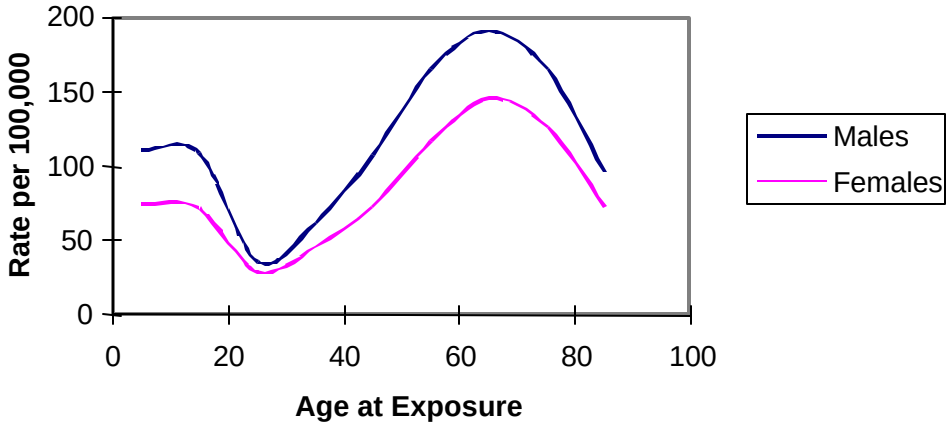
There are limited data on the effects of direct exposure of children to benzene. However, there is some indirect mechanistic evidence to suggest that children may be susceptible to benzene-induced childhood leukemia from *in utero* exposure. There is mounting evidence that key genetic events related to the development of childhood leukemia occur in the developing fetus. In both major forms of infant leukemia, acute lymphocytic leukemia and acute myelogenous leukemia, about 80 percent of *de novo* cases have rearrangements in the *MLL* gene at 11q23 (Ross et al., 1994). The *MLL* gene resembles a homeobox gene and is believed to help regulate the development of the organism and plays an important role in hematopoiesis (Ross et al., 1994). Studies of identical twins who develop leukemia have shown that the genetic change is acquired *in utero* and can be transferred from one twin to another, presumably from transfer of blood cells *in utero* from one twin to another. It has been suggested that

this genetic lesion in an appropriate stem cell is sufficient to cause infant leukemia (reviewed in Alexander et al., 2001). The *MLL* fusion product is rare in cases of *de novo* leukemia among older patients, except following treatment with topoisomerase II inhibiting chemotherapeutic drugs (Ross et al., 1994). Thus, it has been hypothesized that *in utero* exposures to topoisomerase II inhibiting compounds is relevant to childhood leukemia (Ross et al., 1994). Indeed, recent studies have found strong associations with infant leukemia and consumption of dietary topoisomerase II inhibitors (Ross et al., 1996), and with exposure to dipyrone ("Mexican aspirin") and propoxur (the insecticide, Baygon), two chemicals that are suspected to be topoisomerase II inhibitors based on their metabolism to phenolic compounds (Alexander et al., 2001). If this hypothesis proves to be correct, then benzene may also cause infant leukemia via this mechanism, since the major metabolites of benzene, namely phenol, catechol, hydroquinone, 1,2,4-benzenetriol, benzoquinone and trans,trans-muconaldehyde, are all topoisomerase II inhibitors (Hutt and Kalf, 1996; Franz et al., 1996). More research is needed.

Infants and children also may be vulnerable to leukemia induction from benzene because their hematopoietic cell populations are undergoing maturation and differentiation (U.S. EPA, 1998), although this difference may not be as pronounced as for other solid tissues since rapid cell turnover occurs throughout life in the bone marrow.

Our knowledge of radiation-induced leukemia may provide some insight as to the possible age-dependent patterns of leukemia arising from benzene exposures. Many commonalities have been observed between radiation-induced leukemia and benzene-induced leukemia. For example, the pattern of leukemia risk following exposure to ionizing radiation, benzene and chemotherapeutics is similar (OEHHA, 2000a; Finkelstein et al., 2000). Following exposure, leukemia rates rise rapidly within 5 to 15 years then decline to near background levels by about 30 years after exposure (NRC, 1990). Although we do not have information on benzene-induced leukemia for early life exposures, we do have such data from atomic bomb survivors and other radiation-exposed cohorts. This database may provide insight into the age-specific leukemia responses from DNA damage in the marrow. Interestingly, as shown in Figure 1 below, exposures that occur early in life and late in life confer greater excess risk than exposures between the ages of 20 and 45.

Figure 1: Excess Leukemia Mortality by Age at Exposure Following Radiation Exposure (0.1 Sv)



Data from NRC (1990)

e) Mechanistic and metabolic data

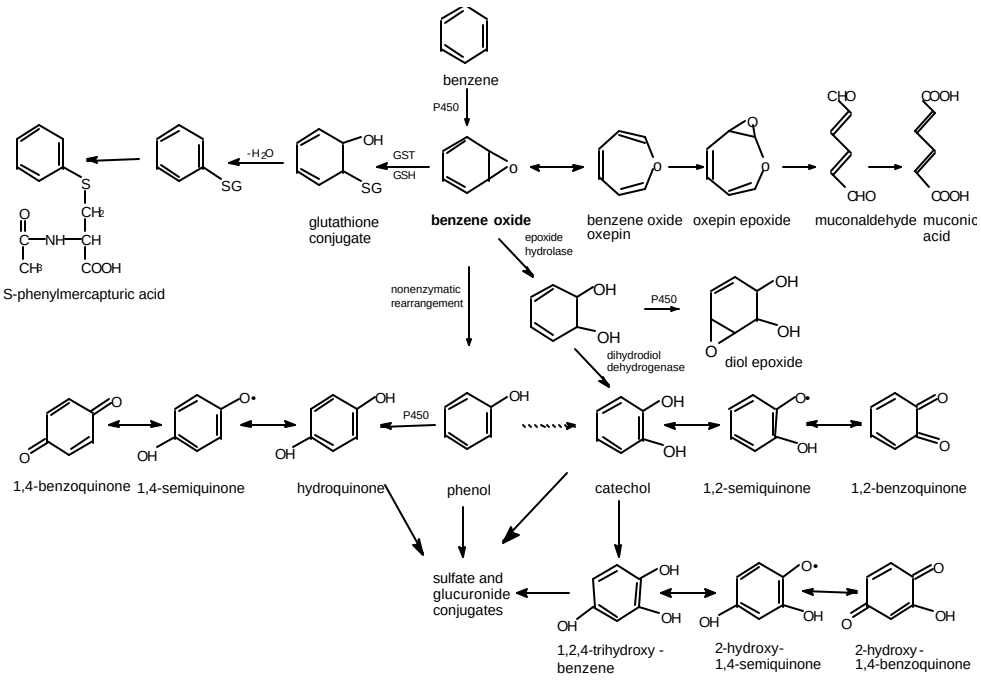
There is strong evidence that metabolism plays a critical role in benzene toxicity (Snyder and Hedli, 1996). For example, competitive inhibition of metabolism by toluene decreases benzene toxicity. Rodents given a partial hepatectomy (Sammett et al., 1979) or mice lacking the CYP2E1 gene (Valentine et al., 1996) had decreased metabolism of benzene and, correspondingly, decreased toxicity. There is no indication that the route of administration has a marked effect on the metabolites formed.

The metabolism of benzene is complex (Figure 2) and has been reviewed elsewhere (Snyder and Hedli, 1996; OEHHA, 2000a). To briefly summarize, benzene is metabolized primarily in the liver by cytochrome P450 2E1 and to a lesser degree by other P450 isozymes to form benzene oxide (or its oxepin) which spontaneously rearranges to phenol. Valentine et al. (1996) confirmed the central role of P450 2E1 by demonstrating that transgenic mice lacking CYP2E1 expression had decreased benzene metabolism, cytotoxicity, and genotoxicity compared to wild type mice.

CYP2E1, whose gene product is cytochrome P4502E1, is not highly expressed early in life (see introductory chapter of this report). Thus, the reduced expression of CYP2E1 in infants may infer a

reduced amount of toxic metabolites formed per unit exposure. However, currently it is unclear to what extent fetal isozymes of cytochrome P450 metabolize benzene. A detailed study of the expression of the other key enzymes in benzene metabolism (e.g., epoxide hydrolase, meyloperoxidase, phenol sulfatases, quinone reductases) would be needed to predict the possible impacts of fetal and infant exposures. In addition, over the course of the first several months of life, there is a maturation of the cytochrome P450 enzyme system and adult isoforms appear as neonatal forms regress. Thus, a young child is capable of producing toxic metabolites of benzene.

Figure 2. Benzene metabolism (from OEHHA, 2000a)



Benzene has been found in maternal and umbilical cord blood (OEHHA, 1997). It also appears likely that metabolites of benzene (formed from maternal metabolism) are transported to the fetus (OEHHA, 1997). Early life expression of important detoxification enzymes such as NADPH-dependent quinone oxidoreductase (NQO1) would likely be an important factor in any benzene-induced DNA damage from fetal- or maternal-formed reactive metabolites. OEHHA is not aware of any studies that have examined the fetal expression of NQO1 in humans, but in rodent liver NQO1 activity is very low in the fetus, but rises to adult levels a few weeks after birth (Hommes et al., 1978).

B. Summary of the Key Animal Studies

Benzene is a multisite carcinogen in rats and mice either by inhalation or oral routes of administration (reviewed in OEHHA, 2000a). Some information on transplacental carcinogenicity comes from studies in which rats were exposed to benzene (200 ppm in air) throughout gestation and lactation, and for an additional 85 weeks after weaning (Maltoni et al., 1983; 1985; 1989). Cancer rates in the offspring were compared to control animals and to their dams, who were exposed to the same concentration of benzene for the same period. Although no statistical analyses were reported, the authors stated that "an enhanced carcinogenic effect of benzene was observed in animals on which treatment was started during embryonal life" and that animals whose exposure began *in utero* had higher incidences of some tumor types than the breeders exposed only as adults (Maltoni et al., 1983; 1985; 1989) (Table 1). No carcinogenicity studies of benzene where exposure was limited to pregnancy were located.

As evident by the incidence data in Table 1, exposures *in utero*, through lactation and adulthood (total 104 wk) caused increased tumor incidences for some tumor type compared to maternal exposures for 85 wk. Among female offspring, Zymbal gland tumors (one of the more consistently responsive tumorigenic sites in rodents) exhibited a 2-fold increase in tumor incidences relative to incidences among the dams. Thus, an increase in overall exposure time by 20 percent resulted in a 2-fold increase in tumor response. It is unclear whether the increased rates are reflective of the increased overall exposure or due to differential susceptibility of the fetus and weanling. A detailed assessment would be required to determine if the increased rates among the rats exposed *in utero* and throughout life are greater than would be predicted by an equivalent 104 wk adult exposure. Although benzene administration to rodents generally does not result in the formation of leukemia, animal models are reasonable predictors of human risk, as cancer potency estimates from human and animal datasets using linear risk models are very similar in magnitude (OEHHA, 2000a).

a) Other Critical Information from Animal studies

(1) Sperm DNA damage

Associations of paternal exposures and childhood leukemia are supported by observations in animals that benzene induces DNA damage in sperm. Mice administered benzene via i.p. injection at seven doses ranging from 0.1 to 1.0 mL/kg-day on five successive days exhibited statistically significant dose-related increases in sperm head abnormalities in dose groups 0.4 mL/kg or higher with a peak effect at 0.6 mL/kg-day (Topham, 1980). Dose-related increases in chromosomal aberrations (breaks, fragments, exchanges) in the sperm were also observed in mice following administration of single oral doses of benzene at 0.25, 0.5 or 1.0 mL/kg relative to controls (Ciranni et al., 1991).

(2) Transplacental genotoxicity

Associations of maternal exposures and childhood leukemia in humans are supported by observations in animal studies that indicate that benzene crosses the placenta and induces DNA damage in the fetus. In mice, hematopoiesis is initiated in the fetal liver on gestational day 10, and peaks on gestational day 12 or 13, which is soon followed by the initiation of hematopoiesis in the bone marrow (OEHHA, 1997). Thus, gestational days 13 to 15 are considered a sensitive period for induction of hematopoietic genotoxicity in the fetal liver. For this reason, most of the relevant studies administered benzene on gestational days 13 to 15.

Increases in benzene-induced micronuclei were observed in fetal liver erythrocytes (polychromatic erythrocytes, PCE) in three studies (Ciranni et al. 1988; Ning et al. 1991; Xing et al. 1992) following exposure of the dams to benzene. A significant increase in fetal liver PCE micronuclei was found in mice given benzene by gavage on gestational day 13 (Ciranni et al. 1988) and on gestational day 14 or 15 (Ning et al. 1991; Xing et al. 1992), but not when given on gestational days 16 to 17 (Harper et al. 1989). Also, two studies reported increases in sister chromatid exchange in fetal cells after dams were administered benzene i.p. (Sharma et al. 1985; Xing et al. 1992).

Table 1. Summary of Carcinogenicity Studies Employing Early-life Exposure of Animals to Benzene

Species	Exposure Concentration	Dose Regimen	Tumor Type	Incidence: Dosed Group (Controls)	Reference
Sprague-Dawley rats (Breeders, 13 wk old)	200 to 300 ppm (see regimen)	200 ppm 4 h/d, 5 d/wk for 7 wk, then 7 h/d, 5 d/wk for 12 wk, then 300 ppm 7 h/d, 5 d/wk for 85 wk	Zymbal gland carcinoma Mammary (malignant and benign combined)	3/54 (1/60) 30/54 (24/60)	Maltoni et al., 1983, 1985, 1989
(Offspring)	200 to 300 ppm	See regimen for Breeders. Offspring exposed *in utero*, during lactation, and for 85 wk (104 wk total). Sacrifice at 150 wk.	Zymbal gland carcinoma Mammary (malignant and benign combined) Nasal carcinoma Hepatoma	14/75 (2/158) m[a] 8/65 (0/149) f[a] 6/75 (11/158) m 35/65 (84/149) f 1/75 (0/158) m 2/65 (0/149) f 2/75 (1/158) m 7/65 (0/149) f	
Sprague-Dawley rats	200 ppm (dose to the dams)	*In utero* from day 12 of gestation and during lactation. 4 hr/d, 5 d/wk for 7 wk, then 7 hr/d, 5 d/wk for 12 wk. Sacrifice 150 wk	Zymbal gland carcinoma Oral cavity carcinoma Hepatoma	4/70 (2/158) m 1/59 (0/149) f 2/70 (0/158) m 6/59 (0/149) f 2/70 (1/158) m 5/59 (0/149) f	Maltoni et al., 1983, 1985, 1989

[a] m, males; f, females

Most studies of transplacental genotoxicity compared effects in the fetus to those in the dam. Two studies found effects in the fetus (liver) and the dam (bone marrow) at similar doses (Sharma et al. 1985; Xing et al. 1992). Two other studies, both using i.p. administration (Ning et al. 1991; Xing et al. 1992) reported an effect in the fetus at a lower dose than in the dam. Thus, mouse fetuses appear to be susceptible to the genotoxic effects of benzene, but sensitivity relative to dams is unclear. Additionally, two studies using oral administration compared benzene-induced genotoxicity in the fetus, dam, non-pregnant female, and adult male. Cirrani et al. (1988) found similar increases in micronuclei of PCEs in virgin females as in pregnant dams and fetuses, but a larger effect in males. Harper et al. (1989) also reported a larger effect in males, a smaller effect in virgin females and, as mentioned above, no effect in pregnant dams or their fetuses, when exposed on gestational days 16 and 17.

(3) Transplacental alteration of hematopoiesis

Epigenetic mechanisms are likely involved in benzene-induced leukemia and include the alteration of hematopoiesis and clonal selection (OEHHA, 2000a). Evidence in animals suggests that exposure to benzene *in utero* alters maturation of lymphocytes, erythrocytes and granulocytes (OEHHA, 1997). The consequences of *in utero* exposure to benzene at air concentrations as low as 5 to 20 ppm can be detected as alterations in cell population numbers and functional properties that in several cases persist into adulthood (Keller and Snyder, 1986; Keller and Snyder, 1988; Corti and Snyder, 1996). Benzene-induced damage during the initial *in utero* stages of hematopoiesis appears to have lasting effects as has been demonstrated for a number of other toxicants (OEHHA, 1997).

V. Conclusions

OEHHA has placed benzene in Tier 2 due primarily to suggestive evidence of associations between benzene exposure and childhood leukemia supported by various lines of evidence from animal studies. Benzene exposures are ubiquitous due to its presence in fossil fuels. Benzene is a known human carcinogen, causing leukemia in worker populations. Although no human cancer studies of benzene-exposed children are available, there is good reason to believe that childhood exposure to benzene

would also contribute to adult-onset leukemias. Also, there is some evidence to suggest that exposure to benzene is associated with childhood leukemia. Paternal exposure to benzene prior to conception in humans has been associated in some studies with increased childhood leukemia, especially of the acute non-lymphocytic type, findings that are supported by observations in animals of benzene-induced DNA damage to sperm. Maternal exposure to benzene in humans also has been associated with increased incidences of childhood leukemia in some studies. These findings are supported by observations in animals of benzene-induced transplacental genotoxicity, altered hematopoiesis, and of carcinogenicity, following exposure *in utero* and continuing until weaning. However, it should be noted that other epidemiological studies that represented a large number of cases of various subtypes of leukemia (Kaatsch et al., 1998) or acute lymphocytic leukemia only (Shu et al., 1999) did not find an association with paternal benzene exposure. Thus, although there is suggestive evidence of an association between benzene and childhood leukemia, a causal relationship would be difficult to establish at this time.

It is difficult to predict whether postnatal exposures to benzene would be more or less likely to initiate leukemia than adult exposures. The impact of maternal-formed metabolites that cross the placenta needs to be considered in such comparisons. Expression of cytochrome P4502E1, the first metabolic step in benzene's metabolism and key to benzene's toxicity, is low in infants suggesting reduced formation of toxic metabolites. However, at several months of age the expression of cytochrome P4502E1 is equivalent to that of an adult. Thus young children are able to metabolize benzene to toxic intermediates. Also, detoxification enzymes such as NQO1 may not be functioning at an early age.

Studies of rodents exposed to benzene *in utero*, through weaning and adulthood exhibited 2-fold higher incidences of tumors of various sites than animals exposed only as adults. Since the two exposure groups received different total doses of benzene, a critical analysis is needed to determine whether the tumor rates observed in the rodents exposed early in life are greater than what would be predicted based on the rates from adult-only exposures. There is no human cancer study available that examines childhood exposures to benzene and lifetime leukemia risk. However, studies of radiation-induced leukemia show that early-life exposures result in greater lifetime risk of leukemia compared to adult exposures. It is not unreasonable to suggest that benzene exposure would cause a similar phenomenon.

Current ambient air levels of benzene (0.85 ppb) (CARB, 2000) are associated with a significant, upper-bound cancer risk in the California population (79 cancer cases per one million exposed), based on the current TAC unit risk factor for benzene. This calculation does not take into account emissions from "hot spots", which would result in additional risk in the immediate vicinity of the source. Thus, it is very important that Cal/EPA assess whether the measures taken under the TAC program to reduce benzene exposure are adequately protective of all major subpopulations, including children.

It should be noted that the current cancer potency values for benzene used in California (and by the U.S. EPA) are based on methods that included children, but do not account for any potential differences in susceptibility (i.e., response). Specifically, cancer potency estimates from the occupational cohort studies are used to estimate the cancer potency of benzene in the general population from continuous exposure. This extrapolation is accomplished using life table methods (NRC, 1990; OEHHA, 2000a). The worker-based potency estimate is applied to the age-specific leukemia rates in the general population starting at 0-5 years of age (which by definition includes childhood and adult leukemias). As has been observed for radiation-induced leukemias, there is evidence to suggest that benzene exposure early in life elicits a stronger carcinogenic response than equivalent exposures of working-age adults. Thus, the current estimate of the cancer potency for benzene may underpredict the risk from early life exposures, and standards based on this potency would not be adequately protective of children.

OEHHA may revisit listing benzene under SB25, particularly if more information on potential differential toxicity between children and adults becomes available.

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